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**Office of Pesticide Programs**  
**Science Policy on**  
  
**The Use of Data on**  
**Cholinesterase Inhibition**  
**for Risk Assessments**  
  
**of Organophosphate and Carbamate**  
**Pesticides**

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## **Tolerance Reassessment Advisory Committee Review Draft<sup>1</sup>**

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Other members of OPP who provided written comments on one or more drafts include Stephanie Irene, Margaret Stasikowski, and Penelope Fenner-Crisp. Internal EPA peer reviewers included Edward Ohanian of the Office of Water, Richard Hill of OPPTS, and Suzanne McMaster, Stephanie Padilla, Robert MacPhail, and Hugh Tilson of ORD. We thank them for their time and efforts at improving this proposal.

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<sup>1</sup> This draft is essentially the same as that presented for SAP review and dated April 30, 1997, except that certain editorial changes have been made, outdated general material removed and an update added to reflect the Agency's presentation to the SAP in June, 1997.

## **I. Introduction**

The purpose of this document is to describe a science policy in the Office of Pesticide Programs (OPP) for the selection of appropriate endpoints for assessing potential risks to humans exposed to cholinesterase inhibiting pesticides. In addition, it will propose a series of steps for conducting risk characterizations for these chemicals.

Regulatory decision making in EPA is described in two major steps, risk assessment and risk management. Risk assessments define the potential adverse health effects which may occur in individuals or populations, while risk management weighs regulatory alternatives and integrates the risk assessment with social, economic, and political concerns. (NAS, 1983).

Risk assessment contains four steps: hazard identification, dose response assessment, exposure analysis, and risk characterization.

Risk assessments for systemic toxicity are generally based on the derivation of reference doses (RfDs)<sup>2</sup>. Reference doses are calculated by dividing the no effect level (NOEL), or other point of departure (e.g., an ED<sub>10</sub> or other benchmark dose), usually for the most sensitive endpoint, called the critical effect, by uncertainty factors (UF). These values are then compared to the potential exposure levels in the risk characterization, which fully describes the nature and extent of the risks posed, and the limitations and uncertainties involved.

Cholinesterase inhibition (ChEI) and cholinergic effects resulting from exposure to organophosphate and carbamate pesticides have long been prominent effects of concern to the USEPA in assessing environmental health risks. The Office of Pesticide Programs (OPP) Reference Dose Tracking Report (3/28/97) lists over 50 Reference Doses for chronic exposure alone based in whole or in part on cholinesterase inhibition. If we consider that acute dietary exposure endpoints, short term and intermediate exposure endpoints are also generally needed for risk assessments, and that ChEI is most often the critical effect for those exposure categories, there are probably over 100 specific risk assessments based on this endpoint for roughly 50 chemicals.

For at least the last ten years, OPP has based these reference doses on the critical effects

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<sup>2</sup> A Reference dose is an estimate, with uncertainty spanning perhaps an order of magnitude, of a daily exposure to human populations, including sensitive subgroups, that is likely to be without appreciable risk of deleterious effects during a lifetime.  $RfD = NOEL/UF$ . Application of uncertainty factors typically involves use of 10 for intra-species differences and 10 for inter-species extrapolation, a total of 100, standard factors for systemic toxicity. Comparable evaluations for acute, short term, or intermediate exposures are derived in exactly the same way.

of plasma, red blood cell, and brain ChE inhibition, or functional effects, and has used the same uncertainty factors, e.g., 10 for inter-species and 10 for intra-species extrapolation, for all of those endpoints. Further, OPP has used statistical significance, rather than a fixed generic difference from baseline, e.g., 20% inhibition, as the primary, but not exclusive determinant of toxicological significance. Both the use of uncertainty factors and this use of statistical significance are consistent with EPA practice for most systemic toxicity endpoints.

### **A. Previous EPA Policy Proposals and SAP/SAB Reviews**

There have been four major external groups in the last 10 years that have been asked by EPA to review proposed science policy positions for this type of neurotoxic effect. One peer review colloquium (US EPA, 1988) and two SAB/SAP meetings (US EPA, 1990, 1993) considered EPA reports in this area. A SAP/SAB review in 1992 of a proposed reference dose on aldicarb also addressed these issues (US EPA, 1992). Each of these groups provided somewhat different recommendations, based in part on the different policy proposals, as well on their differing judgments. The area of greatest divergence among these reports and in these recommendations, involves the interpretation and use of blood measures of ChE inhibition for deriving reference doses.

Two other federal groups have issued general guidelines on neurotoxicity risk assessment that have mentioned this area.

Outside of EPA, two major regulatory groups have published their views of the use of this type of neurotoxicity data. A group of experts, on behalf of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization, have described the evolution of positions in various groups meeting for those bodies on the use of cholinesterase inhibition data (WHO, 1990). A manual of The Department of Pesticide Regulations of the state of California also provides draft guidance on the use of cholinesterase inhibition data in risk assessments for pesticides (Lewis, 1993). The sections quoted below are focused on the discussions related to the interpretation and use of blood measures, and some considerations regarding uncertainty factors and statistical analysis.

#### **1. 1988 RAF Peer Review Colloquium**

In 1988 a Peer Review Panel for the EPA Risk Assessment Forum reviewed an EPA Technical Panel Report on Cholinesterase Inhibition as an Indication of Adverse Toxicologic Effect (June 1988).

On the adversity of ChE inhibition the following consensus conclusion was reported:

"After considerable discussion, the Review Panel agreed with the conclusion of the

Technical Panel that inhibition of brain ChEs is an adverse effect. Statistically significant inhibition of blood ChEs is sufficient indication of a potential adverse biological effect, and reversible effects should be taken as seriously as irreversible effects. Concern however, was expressed that there are no data to support a simple correlation between a particular ChE inhibition level and an observable biological effect."

In response to a question about what level of ChE inhibition constitutes toxicological significance, they concluded: "In general, the Review Panel agreed with the Technical Panel's conclusion that baseline, pre-exposure ChE levels provide the best basis for statistical comparisons." (This was, in part, in contrast to use of a generic value of 20% as a threshold for toxicological significance).

## **2. 1990 SAB/SAP Meeting**

In 1990, an SAP/SAB panel considered a revised EPA Technical Panel report and recommendations. On the issue of adversity of blood measures they concluded:

"The Joint Group expressed doubt about the validity of plasma and red blood cell (RBC) cholinesterase inhibition (ChEI) as indicators of toxicity. Members pointed out that these measures could not be correlated with recognized adverse effects. In fact, such measures may indicate that the organism's defenses against toxicity are intact."

On the issue of uncertainty factors they concluded:

"Base the criteria for adverse effects upon adverse effects. That is, define an adverse effect on the basis of functional (behavioral, electrophysiological) measures, accompanied, where feasible, by morphological indices..."

"Replace the NOAEL/UF strategy with one based on the kinds of dose-consequence data available..." "From these, distill a specified level of ChEI, based on say, a 10% decrement of performance. To the 95% lower bound, attach a UF to yield the RfD."

## **3. 1992 SAB/SAP Meeting**

Another EPA ChE policy report was reviewed by another SAB/SAP Panel in November of 1992 (US EPA, 1993). On the use of blood measures alone for risk assessment, they concluded,

"The Committee reached no simple "yes" or "no" answer on the question of using cholinesterase inhibition, by itself, for risk assessment purposes."

"There was full agreement among the Committee members that blood cholinesterase inhibition is a biomarker of exposure, and that data regarding inhibition of the blood enzymes are often crucial supporting data for confirming exposures and corroborating clinical signs. We recommend that the Agency's policy continue to include the use of blood cholinesterase data in the risk assessment process, in particular in human studies where cholinesterase data from the target tissues of most concern (i.e., brain and peripheral nervous system) are unavailable."

This Committee also emphasized that "The inclusion of biochemical data regarding cholinesterase inhibition with these signs and symptoms is considered essential for the complete hazard evaluation for these compounds." Last, they supported the use of statistically significant brain ChE inhibition for setting reference doses, but noted the importance of regional measures and correlative blood measures.

#### **4. 1992 SAB/SAP Aldicarb RfD Review**

On the next day, in response to a question concerning the use of blood cholinesterase data for aldicarb and in general, another SAP/SAB panel gave the following response.

"The committee felt in general that blood ChEI data are highly relevant to determination of NOELs, NOAELs, and RfDs. As detailed in a separate report, it was felt appropriate to emphasize functional data that are obviously related to toxic effects, that are quantitative and demonstrate a dose response. It was expected that ChE would usually be a sensitive and relevant variable, both as a quantitative predictor, as a measure of exposure *per se*, as an index of the depletion of what may represent a protective buffer or biological site for ChE inhibitors and as a biomarker of effects occurring outside of the central nervous system. Finally, this variable is the only one which is directly comparable from animal studies to human studies. The final consensus was that both cholinesterase data and clinical/functional findings be used where appropriate and that regardless of how derived, RfDs should ensure that there would be no significant cholinesterase inhibition. The committee recommended the submissions contain cholinesterase data but that those consisting solely of cholinesterase data not be considered."

#### **5. June 1997 SAP Meeting**

In June, 1997, the Agency made a presentation to the SAP which included a literature review, a series of case studies, a summary of activities related to methodology of ChE measurement, and a briefing of the Agency's science policy on cholinesterase inhibition for risk assessment. This briefing covered the April 30, 1997 draft of this paper, provided EPA's analysis of the issues and options, and proposed to use a weight of evidence approach considering all of the data that might result in the use of ChE measures in plasma, red blood cells or brain for defining critical effects. The SAP firmly supported the Agency's weight of evidence approach for cholinesterase inhibitors as "reasonable and justified" as long as the data used "are derived from

from rigorous experiments with standardized methods and proper controls.” The SAP also agreed that the Agency should continue evaluating data on cholinesterase inhibition in the peripheral nervous system.

### **B. 1994 FCCSET and 1995 EPA Neurotoxicity Risk Assessment Guidelines**

Both a Federal Coordinating Council for Science, Engineering, and Technology (FCCSET) and EPA have published guidelines for neurotoxicity risk assessment that address in part, the issue of ChE inhibition.

The FCCSET document (US EPA, 1994) rather tersely notes "Inhibition of this enzyme (AChE) in brain may be considered evidence of neurotoxicity, whereas decreases in AChE in blood, which can easily be determined in humans, are only suggestive of a neurotoxic effect."

A proposed EPA Neurotoxicity Risk Assessment Guideline (US EPA, 1995c) also briefly reviews the issue of ChE inhibition. It concludes that "statistically significant decreases in brain cholinesterase inhibition could be considered to be a biologically significant effect" but describes a lack of consensus about "whether RBC and/or plasma cholinesterase represent biologically significant events." An SAB meeting in 1996 (US EPA 1997b) to review the draft guideline was asked to address 2 issues: "the use of blood and/or brain acetylcholinesterase activity as an indication of neurotoxicity for risk assessment"; and "Considering the available data and the state of the science, does the SAB agree with the recommendation that inhibition of RBC and/or plasma cholinesterase can serve only as a biomarker of exposure? (Drs. Pfitzer, Weiss)."

Their response was, "The Committee addressed these two issues together because of their close relationship. The EHC concurred with the findings of previous SAB reviews regarding the consideration of data on the inhibition of RBC and/or plasma cholinesterase. In the absence of clinical signs in humans or animals or the absence of morphological data in animals, the quantitative nature of the inhibition of red blood cell (RBC) and/or plasma cholinesterase inhibition is considered unreliable for assessing significant biological adverse changes, but can be used as a biomarker of exposure. The Committee also recommended that a noted decline in brain ChE should be evaluated by risk assessors in terms of possible effects that are biologically significant, and that the term "statistically significant" needed to be better explicated - perhaps in terms of the benchmark dose or by some measure which reflected information about the distribution of the effect under study. The Committee also suggested that further details concerning reversibility and possible tolerance effects (which could enhance sensitivity to other agents) be provided."

### **C. 1990 Experts for UNEP, ILO, and WHO**

A group of experts, on behalf of the United Nations Environment Programme, the

International Labour Organisation, and the World Health Organization, have described the evolution of positions in various groups meeting for those bodies on the use of cholinesterase inhibition data (WHO, 1990).

From 1967-1982, their review groups used plasma and RBC ChE inhibition in their risk assessment documents, but noted that blood measures were not useful "as an invariable guide to the degree of intoxication present or predicted."

In 1982, they reconsidered their position and focussed on the use of RBC AChE since it contained AChE, and pronounced as biologically significant a "reduction of >20% of pretest levels in the same animals in short duration studies, or in concurrent controls in longer studies." No further rationale for this 20% level is provided.

In 1988, they noted "the correlation between acetylcholinesterase inhibition in erythrocytes and in the nervous system is usually unknown" and found brain levels of ChEI to be of greater value, but noted that RBC ChEI was still better than plasma. They also noted that "*in vitro* kinetic studies may be necessary for pesticides with anti-esterase activity."

Last, they noted concerns about ChE methodology for carbamates, and the adequacy of reporting of assay details, and concluded that "The results obtained in *in vivo* studies should be interpreted cautiously until more satisfactory methods are available."

#### **D. 1993 California DPR Guidance**

Lewis (1993), for the Department of Pesticide Regulation of the state of California, has also written draft guidance on the use of cholinesterase inhibition data in risk assessments for pesticides. While brief, this document contains a detailed review of literature related to the interpretation of changes in ChEI measures in the absence of clinical signs.

While noting the species differences in the amount of AChE in rats and humans, they conclude that blood ChE of any kind will not be regarded as an adverse effect. They cite a number of animal studies to indicate that a wide range of levels of brain ChEI may be associated with overt signs, i.e., 15-80%. They conclude that "if there is statistically significant inhibition of brain ChE inhibition (sic), there is probably some deleterious effect on the neurological system." They note that ChE decreases in peripheral tissues should also be regarded as adverse. After review of studies on variations in ChE measures within and between individuals, they endorse the use of concurrent controls for long term studies, or the use of individual pre-exposure measures for acute and subchronic studies, if available.

While not supporting the general use of blood measures of ChEI for risk assessments, they go on to note a number of instances where that might be done:



first, in animal or human studies where brain ChE was not measured, using the blood measure, plasma or RBCs, which best correlated with brain ChEI in other studies;

second, "if there is strong evidence... that the chemical does not penetrate the blood brain barrier and therefore the cholinergic effects are predominantly peripheral in origin."

They would also use the blood measures if peripheral tissue levels were not available and if the cholinergic effects correlated with the blood measures.

### **E. Discussion**

Until the 1997 SAP meeting, the Agency had been unable to define a consensus policy on cholinesterase inhibition. However, with EPA's proposed policy emphasizing the weight of evidence analysis, the SAP agreed that the Agency was taking a reasonable and scientifically sound approach.

The remainder of this paper consists of a summary science policy statement and more detailed guidance on evaluation of functional data, i.e., clinical signs, human symptoms, and behavioral effects, ChE measures in brain and blood; and a series of steps and analyses to conduct risk characterizations to support completion of the risk assessments and to provide a framework for consistent risk management decisions.

## **II. Science Policy Statement**

A. For an adequate evaluation of a ChE inhibitor, the essential elements of a critical study or a data base should include:

- ! data on clinical signs (and symptoms in humans);
- ! other functional effects related to ChE inhibition;
- ! measurements of CNS and PNS AChE inhibition;
- ! plasma and RBC ChE inhibition.
- ! data on the time of peak functional and biochemical effects.

B. Clinical signs and other behavioral or neurophysiological effects related to cholinesterase inhibition in humans and animals, and symptoms in humans provide direct evidence of adverse effects<sup>3</sup>.

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<sup>3</sup> Adverse effects include alterations from baseline that diminish an organism's ability to survive, reproduce, or adapt to the environment. (U.S. EPA 1995)

Most commonly reported in humans are headache, nausea, and dizziness. Anxiety and restlessness are prominent. Worsening may result in muscle twitching, weakness, tremor, incoordination, vomiting, abdominal cramps, diarrhea. Often prominent are sweating, salivation, tearing, rhinorrhea, and bronchorrhea. Blurred and/or dark vision, and miosis may also be seen. Tightness in the chest, wheezing and productive cough may progress to frank pulmonary edema. Bradycardia may progress to sinus arrest, or tachycardia and hypertension. Confusion, bizarre behavior, and toxic psychosis may occur. In severe poisonings, toxic myocardiopathy, unconsciousness, incontinence, convulsions, respiratory depression and death may be seen. Repeated absorption, but not enough to cause acute poisoning may result in persistent anorexia, weakness, and malaise.  
(U.S. EPA, 1989)

C. Inhibition of acetylcholinesterase in the central nervous system is an indicator of an adverse effect because it interferes with the timely de-activation of neuronal acetylcholine, which prolongs the actions of cholinergic neurons which results in the adverse effects associated with these chemicals.

D. Inhibition of acetylcholinesterase in the peripheral nervous system or at neuroeffector junctions is, by the same mechanism, an indicator of adverse effects on the peripheral nervous system.

E. Blood cholinesterase inhibition<sup>4,5</sup> represents an indirect indicator of adverse effects on the nervous system. While blood measures of ChEI are not adverse in themselves, they are generally the only available estimator of ChEI potential in the peripheral nervous system, since data on ChEI in peripheral nervous tissues or target organs are rarely available. In humans, blood ChEI measures serve as the essential estimators of ChEI potential in both the central and peripheral nervous systems, since neither CNS nor PNS or related organ ChEI measures are available.

F. OPP will use a weight of evidence (WOE) approach to select the appropriate endpoint for risk assessment. This includes analysis and comparison of the dose effect data from all available studies, a description of the strengths, weaknesses, and limitations of the data, identification of data needs that might be needed to refine the data base, and finally the application

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<sup>4</sup> Blood cholinesterase in this document refers to both plasma cholinesterases and red blood cell acetylcholinesterase (AChE).

<sup>5</sup> While human plasma is predominantly butyrylcholinesterase (BuChE), (AChE:BuChE, 1:1000), in rats, plasma contains a considerable amount of AChE (AChE:BuChE, 3:1; males)(Brimijoin, 1991).

of our best scientific judgments. Based on this weight of the evidence analysis for any ChE inhibiting pesticide, OPP may select as critical effects:

- ! clinical signs and other behavioral or neurophysiological effects in humans and animals;
- ! symptoms in humans;
- ! central or peripheral nervous tissue measures of ChE inhibition; or
- ! blood measures of ChE inhibition.

G. There are a number of instances where the use of blood ChE inhibition can be more readily justified as a critical effect for a risk assessment based on the weight of evidence analysis of the available data. Examples include but are not limited to:

1. A pesticide which, based on animal data, exhibits a steep dose effect curve for the development of progressively more severe toxic effects and where blood ChEI is the most sensitive effect;
2. A pesticide for which, the LOELs and NOELs for various indicators of ChEI are essentially the same;
3. An OP for which there is evidence from toxicity, metabolism, or pharmacokinetic studies, or other sources, to indicate that it poorly penetrates the blood-brain barrier such that its potential effects would be expected to be mediated largely through the peripheral nervous system;
4. An OP for which the available human data are judged to be the most critical data for risk assessment and where blood ChEI is the most sensitive effect. In the absence of brain ChE activity measurements, which are not made in human studies, the inhibition of blood ChE activity can serve as an indirect indicator of potential adverse effects in the CNS.
5. When there is a wide disparity in doses between those affecting blood ChE and other parameters and when there is an absence of other data (see I. below).

H. The primary objective of the WOE analysis is to determine the critical effect and calculate a reference dose (RfD) or margin of exposure (MOE)<sup>6</sup>. Evaluation of statistical and toxicological significance<sup>7</sup> and application of uncertainty factors, e.g., 10 for inter-species and 10

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<sup>6</sup> A margin of exposure is the ratio of the no effect level to the exposure level, NOEL/EXPOSURE LEVEL = MOE.

<sup>7</sup> While statistical significance is the primary empirical measure inherent in the experimental design, it is recognized that judgment is important in considering the toxicological

for intra-species extrapolation, will follow the established procedures for assessing potential human risk.

I. Where there are significant uncertainties regarding the available data or the resulting risk characterization, an iterative process for refining the risk characterization should be followed. Refinements of the risk characterizations are intended to provide risk managers with analyses that will allow them to evaluate potential risks, including evaluation of risk mitigation options in a way that is clear, transparent, and consistent. These steps may include:

! Collection of additional data, to address a number of issues such as:

- to provide better dose effect data and to refine NOELs;
- to provide data on PNS ChEI;
- to provide data on metabolism, pharmacokinetics and pharmacodynamics; or
- to provide direct data on exposure routes of interest;

! Refinement of dose response assessments by evaluating LOELs and NOELs for all compartments, and by defining as completely as possible dose effect data for all critical effects and/or compartments

! Repeating the risk characterizations for the expanded data base and refined dose effect data.

### **III. Guidance For Evaluating Chemically Induced ChE Inhibition**

There are 2 major divisions of the nervous system, both of which contain cholinergic pathways that may be affected by cholinesterase inhibitors:

"the peripheral nervous system (PNS) consisting of skeletal muscle, and tissues of the autonomic nervous system, consisting of ganglia of the sympathetic and parasympathetic nervous systems, smooth muscles, cardiac muscle, and glands;" "and ...the central nervous system (CNS), consisting of brain and spinal cord." (Dementi, 1997).

Access of chemicals to the central nervous system is limited by the blood brain barrier. Lacking such a fine barrier, the peripheral nervous system is more accessible to many chemicals. Many of the typical adverse effects of ChE inhibitors may be peripherally mediated.

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significance of very small changes in a data set with small variability, or the lack of statistical significance of large changes. Historical control data or additional analyses may help address such issues. (See also, US EPA 1993, p 15)

## **A. Functional Effects**

Clinical signs and symptoms in humans, clinical signs in animals, and neurobehavioral effects in both humans and animals are the physiological and behavioral effects typically associated with exposure to ChE inhibitors and are most generally regarded as adverse and therefore considered first in defining critical effects. In addition to the common physiological cholinergic signs, many more complex functions may be impaired by ChE inhibition in the peripheral or central nervous system. They may be produced following acute or repeated exposures, and would require exhaustive and specialized testing for complete evaluation (See Dementi, 1997).

Generally, evaluation of clinical signs and behavioral effects depends on the scale of measurement (descriptive versus quantitative), number of subjects, the power of the study and statistical significance, and toxicological significance. The spectrum of effects that can be evaluated in humans is greater than the effects generally studied in animals, but is often limited in available studies of either species. Learning and memory evaluations, for example, are rare, though they are a potential target of ChE inhibitors due to the role of cholinergic systems in these functions. Repeated exposure, due to the development of tolerance for clinical signs, sometimes can fail to produce typical signs of acute toxicity in the presence of extensive changes in neurochemistry (See Dementi, 1997).

Different chemicals may and generally do produce different spectra of clinical signs and behavioral effects. This complexity in part may arise from differences in distribution between the CNS and PNS, differential binding in those compartments, or differential interactions with the 2 major types of cholinergic receptors, muscarinic and nicotinic receptors. The nature and temporal pattern of effects may also depend on the rate of exposure and whether metabolic activation is needed.

Due to our limited understanding of the precise relationships between behavioral and neurochemical measures of ChEI, we seek both types of measures for an broader perspective on potential effects. This has been a general tenet in testing for neurotoxicity, i.e., to examine effects at different levels of organization of the nervous system, and mirrors the broad efforts in toxicology at proceeding from identification of hazards to an understanding of the biochemical mechanism of action. Since the generally accepted mechanism of action of these chemicals is the neurochemical inhibition of AChE, these measures are the presumptive choice as the biochemical correlate of the functional effects.

## **B. Neurochemical Effects**

### **1. CNS AChE Inhibition**

Data on CNS acetylcholinesterase inhibition typically come from animal studies, in which whole brain homogenates (or brain regions) are assayed periodically or, more commonly, at the end of exposure. Statistically significant decreases in brain ChE are generally considered toxicologically significant because they define a change in nervous system functions, by blocking the degradation of ACh and prolonging the action of the nerve cells. This effect in the brain and peripheral nervous system is the generally accepted mechanism by which the expected overt and adverse effects are caused. Brain cholinesterase inhibition provides direct evidence of adverse effects on the nervous system and may be used to define a critical effect.

As noted earlier, concomitant evaluation of clinical signs, behavioral effects, and blood ChE inhibition are considered essential for an overall evaluation of a pesticide. Reductions in brain ChE activity may or may not be accompanied by overt clinical signs or symptoms because many behavioral and physiological effects, including death, may be predominantly mediated through the peripheral nervous system. Further, the CNS functions potentially affected may not be sufficiently assessed. Whole brain measurements may also mask changes in specific brain regions associated with particular functions (e.g., hippocampus and memory). Time of assessment as well as other factors generally affecting these neurotoxicity studies may also contribute to the lack of concordance.

## **2. Peripheral Nervous System and Neuroeffector ChE Inhibition**

As with the CNS, inhibition of acetylcholinesterase in the PNS and neuroeffector junctions is an indicator of adverse effects in the PNS. Although PNS ChEI has rarely been evaluated in toxicological studies submitted to EPA, there have long been recognition of the potential value of such data, and there is merit in developing and standardizing techniques to assess this compartment. Many of the adverse signs and symptoms associated with exposure to ChE inhibiting pesticides, e.g. diarrhea, excess salivation, are peripherally mediated.

## **3. Blood ChE Inhibition**

Blood cholinesterase inhibition provides direct evidence of exposure but only indirect evidence of neurotoxicity or adverse effects. There are many reasons why the blood measures of ChE should be considered as an appropriate endpoint for derivation of reference doses as a matter of prudent science policy. Blood measures are generally the only available estimator of ChEI potential in the peripheral nervous system, so while they are not adverse in themselves, they can be a unique indirect measure of potential PNS toxicity. In humans, neither CNS nor PNS ChEI measures are available, so blood ChEI measures serve as the essential estimators of both central and peripheral nervous system ChEI potential.

Blood ChEI is not only a measure of exposure, but also a measure of a pesticide's ability to bind to AChE. This is because the binding of a pesticide to the neural and blood enzyme AChE

is essentially the same, or in the case of BuChE at least somewhat similar. Pharmacokinetically, both the blood and the peripheral nervous system are outside the CNS. So for pesticides with limited penetration of the blood-brain barrier, blood ChE measures may be much better indicators of PNS ChEI activity than brain measures.

RBC AChE is typically regarded as all AChE in humans and animals, and plasma ChE is often viewed as BuChE. Since in neurons AChE is the active ChE, it is naturally considered that RBC AChE more closely reflects neuronal activity, and that plasma BuChEs appear less relevant. But the composition of plasma ChEs vary widely between humans and rats<sup>4</sup>. Plasma ChEs in rats may contain mostly AChE, the neuronal form. Thus, for rat studies, the most common test species, a significant portion of plasma ChEI may be due to AChEI, the form most directly relevant to neural functions. In other cases, for unclear reasons, empirical correlations between plasma and brain ChE may exceed those between RBCs and brain ChE (see Dementi, 1997; also ACRA Case study 1).

Demonstration of inhibition of both plasma and RBC ChEs in workers (in the absence of signs, symptoms, or other behavioral effects) are rightly considered as providing sufficient grounds for companies/agencies to remove workers from the exposure environment.

As for CNS effects, functional evaluation of the peripheral nervous system may be quite limited, e.g., for cardiovascular effects.

Further, measurement of ChE activity at peripheral target sites is rarely done. So, for most pesticide data bases, the only available estimate of ChE inhibition in the PNS will be the blood measures of ChE.

Limited reporting of methodological details or of assay conduct are very common in available studies. In some cases, the methods as used may underestimate red blood cell AChEI. Increased variability (coefficients of variation) related to assay conduct can decrease the sensitivity of the assay, i.e., will require larger levels of inhibition to achieve statistical significance. Further details and current efforts in OPP directed at these issues are discussed in other parts of this package (Hamernik, 1995).

Many other factors can influence the observed pattern of blood ChE inhibition. These include a) the time course and reversibility of inhibition, b) time of measurement with respect to the time course of inhibition, c) whether or not the inhibitor is metabolically activated, d) analytical methodology used, and e) whether comparisons are made with pre-exposure measurements in the same subjects or separate control groups (See Dementi for further discussion).

#### **IV. Guidance for Risk Assessments of Cholinesterase Inhibitors**

**A. Dose Response Assessment: Weight of Evidence Analysis for selection of critical effects.**

A weight of the evidence approach for evaluation of any ChE inhibitor should consider all of the available data from animal and human studies, and human exposures to identify the hazards and the exposure levels at which they occur. First the individual studies are evaluated, then all studies and their relation to one another are examined in concert.

**1. Analysis of Individual Studies**

Each study may include ChE measures in blood and brain, PNS (though rarely), clinical signs and symptoms (from humans, if available), and other functional data. Following critical evaluation of the validity of a study, No-observed-effect-levels (NOELs) and/or lowest-observed-effect-levels (LOELs) are determined. The evaluation of each study involves consideration of the study design including dose spacing, the analytical and behavioral methods used, whether pre-exposure data were obtained, the conduct of the study, the statistical analysis and significance (both statistical and biological) of the results, the slope of the dose response and dose effect curve(s), the consistency of the findings within the study when repeated measures are taken, and the relation of the effects seen to one another.

**2. Analysis of the Data Base**

When evaluating the entire database, consistency of LOELs and NOELs for clinical signs, behavioral effects, ChE inhibition in the various compartments, in different studies within a species, across species, across durations of exposure, and across routes all may contribute to the weight of the evidence for the critical endpoints needed. Pharmacokinetic data may also be important.

**3. Selection of the Critical Effect**

Typically, a critical effect level is selected for a route and duration of exposure that represents the most sensitive effect seen. Based on considerations of the weight of the evidence from all of the studies as a group, this level may or may not be the lowest one in which an effect was seen. Valid and reliable human data, when available, take precedence.

An RfD for chronic dietary exposures is then derived by division of the critical effect NOEL by uncertainty factors to account for potential inter-species (animals to humans) and intra-species (among all people at risk) variability. For acute, short term, and intermediate exposures, the critical effect NOEL is identified from the appropriate study(ies). This is typically the end of the dose response assessment stage in the risk assessment process.



## **B. Risk Characterization**

Risk characterization has been described as the interface between risk assessment and risk management (US EPA, 1995). This stage involves the integration of the exposure analysis with the reference dose or critical effects data to derive estimates of the potential risks for each exposure and population of concern. This stage may include both an iterative approach to risk assessment and presenting multiple risk descriptors. It also should describe the limitations and uncertainties in all of the earlier steps in the risk assessment process. What follows is guidance for risk characterizations for ChE inhibitors. It is an iterative process using multiple RfDs (or other available data) as risk descriptors to facilitate risk management decisions.

After analysis of the exposure data, calculate margins of exposure or compare the anticipated exposure levels to the reference dose for each situation of interest. A margin of exposure (MOE) is a comparison made by dividing the NOEL by an anticipated human exposure level. This is the procedure used for acute, short term and intermediate exposures, by tradition. Comparison of exposures to the RfD or calculated MOEs are then used in risk characterizations to evaluate exposures of concern. If reviews of RfDs or MOEs lead to significant concerns, an iterative sequence of actions may be considered to refine the risk assessment (including refining the exposure issues which is not described here).

### **1. Collect additional data**

A comprehensive risk assessment should describe the dose effect curves for each compartment (plasma, RBC, and brain) or other endpoints (e.g., clinical signs) and would allow estimation of e.g., ED10s, so that simple and consistent comparisons could be made between different compartments. Given that toxicology guideline studies usually have 3 doses, and that one dose is usually chosen to be a NOEL, the dose effect relationship may be quite difficult to ascertain. Limited knowledge may thus result in NOELs and associated RfDs that over or underestimate the true potency of the pesticide. In this situation, data on intermediate dose levels or replication of a key finding may be needed to better define the dose effect curves and to more clearly establish critical effect levels. Benchmark dose estimation or other curve fitting may be helpful in some cases.

In some cases, study by the dermal or inhalation route may provide a better and direct means of risk assessment for those routes of potential exposure, reducing or eliminating the uncertainties that may arise from route to route extrapolation.

When most or all LOELs for different measures are seen within a narrow dose range, as in our experience is generally the case, there is greater confidence in the selection of their associated NOELs for use in the derivation of RfDs or MOEs. And there will be less debate about the adversity of the endpoints if direct measures are involved. On the other hand, if significant

inhibition in blood compartments is seen at much lower doses than in other ChE compartments or than in functional measures, there is less coherence in the data set, and there may be more concern about the selection of the critical effects. This may reflect data seen in one study, one species, or be a consistent finding across the database for a chemical.

In some cases, direct measurement of ChEI in peripheral neural or neuroeffector target tissues may be considered. If those tissues are assayed, they would provide direct evidence of ChEI in peripheral tissues, and would potentially be more relevant than the indirect measures of the blood. While current methods for measuring ChEI in peripheral tissues have not been required and may pose some technical difficulties, they offer a potential scientific means to clarify the meaning of blood ChE measures in animals.

## **2. Broaden the scope of critical doses and effects examined and the risk characterization for the expanded data base and dose effect data**

Expand the analysis beyond the use of one critical RfD, by defining RfDs for all compartments, and as completely as possible defining the dose effect data for all critical effects and/or compartments. An attempt to illustrate this idea is provided in Figure 1. This graph plots Exposure incidence (as a % of exposures) against dose in mg/kg. Reference doses for blood ChEI, brain ChEI, and clinical signs are indicated by broad bars. A theoretical exposure distribution is plotted as a curve. Risks of any exposure and for each effect then can be seen visually. This approach can then be used for different exposure distributions, which may represent different commodities, or different application rates, etc. One could also graph dose effect curves similarly for comparisons, if exposures were high enough (not common). A similar approach could be generated to evaluate margins of exposure.

The relationship between exposures and different effects, can be one factor in defining the level of concern for a pattern of toxicity. For example, exposure to a chemical at levels greater than an RfD of 0.01 mg/kg based on, e.g., blood measures may be of greater concern when the RfD based on brain measures is only 3 times that level, than when the RfD based on brain measures is at 50 times that level.

Other critical factors in this broader description of the pattern of observed toxicity may include the nature and severity of effects seen; the slope of the dose effect curves for different effects, and the completeness of the effects evaluated. Other factors important to consider in the total data base are the number of human incidents reported, and the scope of the effects evaluated. Last, the strengths and weaknesses in the data base should be summarized and the uncertainties in defining the critical effects should be clearly documented.

## **3. Evaluate exposures in terms of risk characterization.**

The objective of the expanded risk characterization is to provide as detailed a means as possible for describing the relation between exposures and ChE-related effects of all kinds in qualitative and quantitative terms. This in turn is aimed at providing risk managers with analyses that are clear and transparent and that will serve as a basis for defining consistent risk management decisions.

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